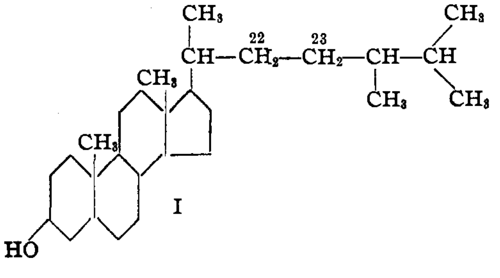
[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY OF PRINCETON UNIVERSITY]

α-Sitosterol

BY EVERETT S. WALLIS AND E. FERNHOLZ[1]

It can now be stated with a fair degree of certainty that the structural formulas of cholesterol, ergosterol and stigmasterol are well established. Our knowledge, however, of the most common plant sterol, sitosterol, is still unsatisfactory. This is due to the fact that sitosterol is in reality a mixture of several sterols, and that their separation is effected with great difficulty. This observation was first made by Anderson, Shriner and Burr.[2] These investigators showed that sitosterol obtained from various sources is not a homogeneous substance but that it contains dihydrositosterol and at least three other sterols. They named these latter compounds α, β- and γ-sitosterol. γ-Sitosterol, which is the least soluble, was isolated by them in nearly a pure state. The purification of β-sitosterol was not so complete. The α-sitosterol was obtained only in a very impure condition; no satisfactory method for its purification was discovered.

Recently certain information has become available regarding the constitution of β- and γ-sitosterol. Bengtsson[3] has compared derivatives of β-sitostanol with stigmastanol. From his experiments he has come to believe that these compounds are identical, and that, therefore, β-sitosterol is in reality 22-dihydrostigmasterol (I).



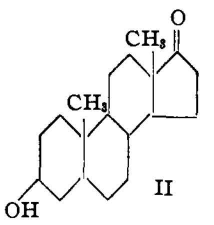
I

This conclusion has indeed a high degree of probability.

A valuable contribution to our knowledge of γ-sitosterol has been made recently by Oppenauer.[4] This investigator prepared dehydroandrosterone (II) from sitosterol If we assume that a sufficiently pure γ-sitosterol was used in these experiments, this fact proves that the ring system of both β- and γ-sitosterol is the same, and that they differ only in the structure or stereochemical arrangement of the side chain.

In this connection mention should be made also of certain other observations. Bonstedt[5] has reported that γ-sitostane and stigmastane, when mixed together, give a strong depression of the melting point. This fact supports the idea that β- and γ-sitosterol are different compounds, and not more or less impure specimens of the same substance. Anderson[2] expresses the opinion that the different sitosterols are isomers.

In this paper we wish to report certain experiments which show that such a conclusion cannot be reached for the most soluble fraction of the sitosterol complex, called by Anderson and coworkers,[2] α-sitosterol.[6] From recrystallization of 500 g. of a sitosterol obtained from wheat germ oil we separated 36 g. of a material rich in α-sitosterol. A method of purification of this material had to be developed since Anderson and his co-workers[2] were unsuccessful in effecting a separation by crystallization of the acetates, and by bromination. Preliminary experiments showed that this could be accomplished by means of a fractional crystallization of the corresponding benzoates. Accordingly this mixture of sterols (36 g.) was converted into the corresponding benzoates. By a single crystallization 23 g. of a benzoate, m. p. 144°, which was mainly β-sitosteryl benzoate, was removed. The α-sitosteryl benzoate remained in the mother liquor. This more soluble benzoate was then hydrolyzed and converted into the corresponding *m*-dinitrobenzoate. By fractional crystallization two new dinitrobenzoates were isolated in a pure state. The least soluble α_1-sitosteryl *m*-dinitrobenzoate was obtained in a yield of 1 g. The more soluble α_2-sitosteryl *m*-dinitrobenzoate, which seems to be the more plentiful constituent, was obtained only in a yield of 0.4 g. It is possible that other



II

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[1] Merck Fellow in Organic Chemistry.

[2] R. J. Anderson, R. L. Shriner and G. O. Burr, THIS JOURNAL, **48**, 2987 (1926); see also Anderson and Shriner, *ibid.*, **48**, 2987 (1926); Anderson, *ibid.*, **46**, 1450 (1924).

[3] B. E. Bengtsson, *Z. physiol. Chem.*, **237**, 46 (1935).

[4] R. V. Oppenauer, *Nature*, **135**, 1039 (1935).

[5] Bonstedt, *Z. physiol. Chem.*, **205**, 137 (1932).

[6] Burian [*Monatsh.*, **18**, 551 (1897)], who also observed the difference in the solubility of this fraction from the bulk of the sitosterol, named it para-sitosterol.

sterol *m*-dinitrobenzoates were present in the mother liquors.

The properties of α_1- and α_2-sitosterol and their derivatives are given in the table.

Derivative	α_1-Sitosterol M. p., °C.	α_1-Sitosterol $[\alpha]^{25}_D$	α_2-Sitosterol M. p. °C.	α_2-Sitosterol $[\alpha]^{25}_D$
Sterol	166	− 1.7	156	+ 3.5
Acetate	137	+29	126	+17
Benzoate	172	+42	166	+27
m-Dinitrobenzoate	222	+37	206	+26

We also wish to report certain other important observations. These two new sterols are *not* isomers of β- and γ-sitosterol. α_1-Sitosterol is an isomer of stigmasterol, $C_{29}H_{48}O$, and α_2-sitosterol is in all probability a homolog, $C_{30}H_{50}O$. Titration with perbenzoic acid shows that two double bonds are present in both sterols. Both sterols give the same color reaction (Liebermann). The final color is a dark blue with a reddish tint. The Salkowski reaction for both α_1- and α_2-sitosterol is similar to that of ergosterol; the sulfuric acid layer becomes colored, while the chloroform stays colorless. The opposite is true for cholesterol, γ-sitosterol, and stigmasterol; α_1- and α_2-sitosterol are precipitated by digitonin.

Experimental Part

Five hundred grams of sitosterol,[7] isolated from wheat germ oil (m. p. 134–137°, $[\alpha]^{20}_D$ −30° in chloroform), was subjected to a systematic crystallization from a mixture of alcohol and benzene, and from alcohol. The most soluble fractions were collected. They had a total weight of 36 g., and melted unsharply at 141°, $[\alpha]^{20}_D$ −24°.

The shape of these crystals is different from those of the main portion. They are small needles. β- and γ-sitosterol crystallize in large leaflets.

This material (36 g.) was dissolved in 150 cc. of pyridine and to the solution was added 30 cc. of benzoyl chloride. The mixture was kept on a boiling water-bath for an hour. The benzoate, so formed, was taken up in ether, and recrystallized from a mixture of benzene and alcohol. Twenty-three grams of a benzoate was obtained in well-defined leaflets which melted at 144°. This is mainly β-sitosteryl benzoate. Ten grams of a crude benzoate, m. p. 115–140°, was also isolated from the mother liquors. This latter fraction was hydrolyzed with 5% alcoholic potassium hydroxide solution. It yielded 7.5 g. of a sterol which melted unsharply at 150°. This sterol was dissolved in 50 cc. of pyridine and treated with 7 g. of *m*-dinitrobenzoyl chloride. The mixture was kept on a boiling water-bath for one hour. The dinitrobenzoate was taken up in ether, and the solution decolorized with animal charcoal. On evaporation of the solvent, 10.8 g. of material, m. p. 170–180°, was obtained.

Isolation of α_1-Sitosteryl *m*-Dinitrobenzoate.—The crude dinitrobenzoate described above was dissolved in 100 cc. of hot ethyl acetate. The solution was allowed to cool; 3.4 g. of yellowish brittle plates crystallized, m. p. 197–207°. This material was recrystallized from the same solvent until the melting point and rotation were constant. The pure crystals melted at 222°. 25.8 mg. dissolved in 2 cc. of chloroform solution gave α^{24}_D +0.48; $[\alpha]^{24}_D$ +37.2°.

Anal. Calcd. for $C_{36}H_{50}N_2O_6$: C, 71.24; H, 8.31; N, 4.62. Found: C, 71.16; H, 8.31; N, 4.78.

α_1-Sitosteryl *m*-Dinitrobenzoate Dioxide.—Twenty-five and eight-tenths milligrams of the above dinitrobenzoate was kept for three days at 0° with an excess of a chloroform solution of perbenzoic acid. The substance consumed 1.33 mg. of oxygen (theoretical amount for 2 atoms of oxygen is 1.36 mg.). The chloroform solution was washed with sodium carbonate solution. The dioxide was recrystallized from a mixture of benzene and petroleum ether. Small needles were obtained which melted at 209–212°.

Anal. Calcd. for $C_{36}H_{50}N_2O_8$: C, 67.67; H, 7.89; N, 4.39. Found: C, 67.70; H, 8.00; N, 4.44.

α_1-Sitosteryl Benzoate.—The above *m*-dinitrobenzoate was hydrolyzed and the free sterol so obtained was dissolved in pyridine and treated with benzoyl chloride. The mixture was kept on a boiling water-bath for one hour. After working up the product in the usual manner a benzoate was obtained which crystallized from alcohol and benzene in the form of flat needles; m. p. 168–172°; $[\alpha]^{27}_D$ +41.8° (28.2 mg. in 2 cc. chloroform solution gave α^{27}_D +0.59°).

Anal. Calcd. for $C_{36}H_{52}O_2$: C, 83.68; H, 10.15. Found: C, 83.93; H, 10.16.

α_1-Sitosterol.—The pure benzoate (m. p. 172°) was hydrolyzed by refluxing it for one hour with a 5% alcoholic solution of potassium hydroxide. The sterol was then precipitated by the addition of water. Purification was accomplished by crystallization from alcohol. Needles were obtained which melted at 164–166°; $[\alpha]^{28}_D$ −1.7° (23.6 mg. in 2 cc. of chloroform solution gave α^{28}_D −0.02°). α_1-Sitosterol is precipitated by digitonin.

Anal. Calcd. for $C_{29}H_{48}O$: C, 84.38; H, 11.73. Found: C, 83.8;[8] H, 11.5.

α_1-Sitosteryl Acetate.—This acetate was prepared by heating a sample of α_1-sitosterol for one hour on the water-bath with acetic anhydride. The acetate crystallized on cooling. Recrystallization from alcohol gave leaflets which melted at 137°; $[\alpha]^{28}_D$ +28.6° (27.3 mg. in 2 cc. of chloroform solution gave α^{28}_D +0.39°).

Anal. Calcd. for $C_{31}H_{50}O_2$: C, 81.87; H, 11.09. Found: C, 81.95, 81.68; H, 10.98, 11.05.

α_2-Sitosteryl *m*-Dinitrobenzoate.—The mother liquor obtained from the first crystallization of α_1-sitosteryl *m*-dinitrobenzoate was concentrated, and alcohol was added until needles started to crystallize from the boiling solution. The material so obtained (m. p. 180–190°) was recrystallized several times from ethyl acetate and alcohol, and from acetone until the melting point, and rotation re-

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(7) The sitosterol used in these experiments was purchased from the University of Iowa.

(8) As is so often the case with sterols, α_1-sitosterol seems to retain a small amount of solvent of crystallization. This is very difficult to remove completely and lowers the value for the percentage of carbon.

mained unchanged. The yield of pure α_2-sitosteryl *m*-dinitrobenzoate was only 0.4 g. An attempt to increase the yield by recrystallization of the material in the mother liquors was unsuccessful.

α_2-Sitosteryl *m*-dinitrobenzoate melted at 206°. It is much more soluble than α_1-sitosteryl *m*-dinitrobenzoate, and forms needles which are white with a yellowish tint. It gives a depression of the melting point when mixed with the α_1-*m*-dinitrobenzoate; $[\alpha]^{30}\text{D}$ +26.4° (24.2 mg. in 2 cc. of chloroform solution gave $\alpha^{30}\text{D}$ +0.32°).

Anal. Calcd. for $C_{37}H_{52}N_2O_6$: C, 71.57; H, 8.45; N, 4.51. Found: C, 71.70, 71.71; H, 8.24, 8.20; N, 4.81.

Titration with Perbenzoic Acid.—Twenty-four and two-tenths milligrams took up 1.38 mg. of oxygen on standing for four days at 0° with an excess of perbenzoic acid. This is equivalent to 2.26 atoms of oxygen. In another experiment 34.4 mg. took up 1.65 mg. of oxygen on standing for two days at 0°. This is equivalent to 1.82 atoms of oxygen. These values are not as good as in α_1-sitosteryl *m*-dinitrobenzoate. They do show, however, the presence of two double bonds.

α_2-Sitosterol.—The *m*-dinitrobenzoate described above was hydrolyzed on the water-bath with a 5% alcoholic solution of potassium hydroxide. The free sterol was recrystallized from alcohol and petroleum ether.

α_2-Sitosterol melts at 156°. It is soluble in the common solvents; $[\alpha]^{25}\text{D}$ +3.5° (22.6 mg. in 2 cc. of chloroform solution gave $\alpha^{25}\text{D}$ +0.04°).

Anal. Calcd. for $C_{30}H_{50}O$: C, 84.43; H, 11.82. Found: C, 84.40; H, 11.91.

α_2-Sitosteryl Benzoate.—α_2-Sitosterol was dissolved in pyridine and treated with benzoyl chloride. The solution was kept on the water-bath for one hour. The product was worked up in the usual manner. The benzoate was purified by recrystallization from a mixture of benzene and alcohol.

α_2-Sitosteryl benzoate is very soluble in benzene, and sparingly soluble in alcohol. It forms needles which melt at 164–166°. The melting point is not depressed by α_1-sitosteryl benzoate. $[\alpha]^{26}\text{D}$ +27.4° (25.6 mg. in 2 cc. chloroform solution gave $\alpha^{26}\text{D}$ +0.35°).

Anal. Calcd. for $C_{37}H_{54}O_2$: C, 83.71; H, 10.26. Found: C, 83.59, 83.39; H, 10.19, 10.32.

α_2-Sitosteryl Acetate.—α_2-Sitosterol was converted into its acetate by heating with acetic anhydride for one hour in a boiling water-bath. The acetate was recrystallized from alcohol. It forms small leaflets which melt at 124–126°. It is soluble in most solvents, but only sparingly soluble in methyl alcohol $[\alpha]^{27}\text{D}$ +16.5° (24.2 mg. in 2 cc. of chloroform solution gave $\alpha^{27}\text{D}$ + 0.20°).

Anal. Calcd. for $C_{32}H_{52}O_2$: C, 81.98; H, 11.19. Found: C, 81.75, 81.83; H, 11.07, 11.28.

We wish to express our thanks to Merck and Company, Inc., Rahway, N. J., for all the analyses in this article and for a grant-in-aid for this work.

Summary

Two new double unsaturated sterols have been isolated in a pure state from the most soluble fraction of the sitosterol complex obtained from wheat germ oil and formerly named by Anderson α-sitosterol.

The acetates, benzoates and *m*-dinitrobenzoates of these two sterols have been prepared and characterized.

α_1-Sitosterol is an isomer of stigmasterol.

α_2-Sitosterol is probably a homolog.

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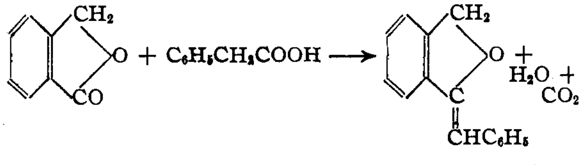
The Synthesis of Benzalphthalane

BY SAMUEL NATELSON AND AARON PEARL

In a recent paper[1] the relationship between benzalphthalide and certain naturally occurring materials was pointed out and a procedure for its conversion to compounds related to this series was demonstrated.

Since the hitherto unknown benzalphthalane offered promise of becoming a valuable intermediate in this field of research, its synthesis appeared desirable. An unsuccessful attempt was made to obtain this product by condensation of phthalide with phenylacetic acid as follows



In this reaction carbon dioxide and water, along with large yields of phthalide and toluene, were formed. Since phenylacetic acid under similar conditions does not evolve carbon dioxide, benzalphthalane was probably the initial reaction product but immediately hydrolyzed in accordance with the equation

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(1) Natelson and Gottfried, THIS JOURNAL, **58**, 1432 (1936).